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Regulation of Nitric Oxide–Sensitive Guanylyl Cyclase

Cyclic GMP Phosphodiesterases and Regulation of Smooth Muscle Function

Structure, Regulation, and Function of Mammalian Membrane Guanylyl Cyclase Receptors, With a Focus on Guanylyl Cyclase-A

Cyclic GMP–Dependent Protein Kinases and the Cardiovascular System: Insights From Genetically Modified Mice

Regulation of Gene Expression by Cyclic GMP

Explaining the Phenomenon of Nitrate Tolerance

*Rudi F. Busse, Editor*

## Regulation of Gene Expression by Cyclic GMP

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**Abstract**—Cyclic GMP, produced in response to nitric oxide and natriuretic peptides, is a key regulator of vascular smooth muscle cell contractility, growth, and differentiation, and is implicated in opposing the pathophysiology of hypertension, cardiac hypertrophy, atherosclerosis, and vascular injury/restenosis. cGMP regulates gene expression both positively and negatively at transcriptional as well as at posttranscriptional levels. cGMP-regulated transcription factors include the cAMP-response element binding protein CREB, the serum response factor SRF, and the nuclear factor of activated T cells NF/AT. cGMP can regulate CREB directly, through phosphorylation by cGMP-dependent protein kinase, or indirectly, through activation of mitogen-activated protein kinase pathways; regulation of SRF and NF/AT by cGMP is indirect, through modulation of RhoA and calcineurin signaling, respectively. Downregulation of the RNA-binding protein HuR by cGMP leads to destabilization of guanylate cyclase mRNA, but this posttranscriptional mechanism may affect many more cGMP-regulated genes. In this review, we discuss the role of cGMP-regulated gene expression in (patho)physiological processes most relevant to the cardiovascular system, such as regulation of vascular tone, cardiac hypertrophy, phenotypic modulation of vascular smooth muscle cells, and regulation of cell proliferation and apoptosis. (*Circ Res.* 2003;93:1034-1046.)

**Key Words:** cyclic GMP ■ transcription ■ mRNA stability ■ translation ■ gene expression

Cyclic GMP is generated by cytoplasmic soluble guanylate cyclases (sGCs), which are activated by nitric oxide (NO), and by receptor guanylate cyclases (rGCs), which are activated by natriuretic peptides [atrial natriuretic peptide (ANP) or B- and C-type natriuretic peptides (BNP and CNP); Figure 1].<sup>1–3</sup> cGMP effector proteins include cGMP-dependent protein kinase (PKG) I and II, cyclic nucleotide-regulated ion channels, and phosphodiesterases (PDEs), which hydrolyze cGMP and/or cAMP.<sup>1</sup> PKG is the major intracellular cGMP target in many cell types, but high cGMP concentrations can cross-activate cAMP-dependent protein kinases (PKA).<sup>3,4</sup> PKG I is highly expressed in platelets,

smooth muscle, glomerular mesangial cells, cardiomyocytes, and many endothelial and neuronal cells.<sup>3,5–9</sup> PKG II is encoded by a different gene with more limited expression<sup>3</sup>; throughout this article PKG will refer to PKG I. The importance of cGMP signaling for the cardiovascular system has been demonstrated in knockout mice lacking either PKG, neuronal, or endothelial NO synthase isoforms (nNOS and eNOS), ANP, or its rGC-A receptor.<sup>2,10,11</sup>

NO can regulate gene expression by multiple, including cGMP-independent mechanisms, as recently reviewed.<sup>12,13</sup> We will concentrate on cGMP-mediated regulation of gene expression defined by the following criteria: the effects

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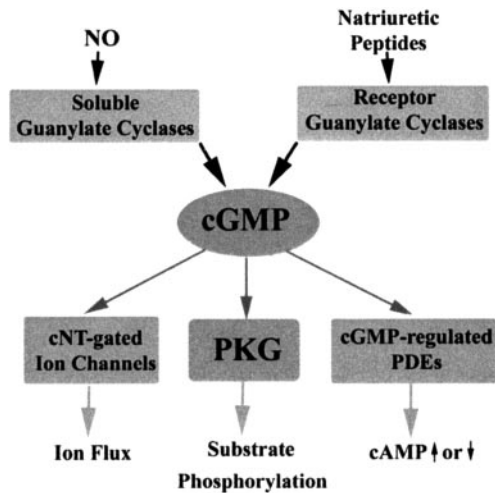
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**Figure 1.** Cyclic GMP signaling pathway. Cyclic GMP is synthesized by soluble guanylate cyclases in response to NO or by receptor guanylate cyclases, which are activated by natriuretic peptides. Depending on the cell type, cGMP has several intracellular targets in addition to cGMP-dependent protein kinases (PKG).

should be observed with activators of sGCs or rGCs at physiologically relevant concentrations; the effect of NO or natriuretic peptides should be blocked by sGC inhibition or an appropriate rGC antagonist, respectively; and effects should be mimicked by membrane-permeable cGMP analogues. At a minimum, we will consider changes in mRNA levels induced by an activator of sGC or rGC that are mimicked by a membrane-permeable cGMP analogue. cGMP-mediated regulation of gene expression can be attributed to the action of PKG, if it is prevented by specific PKG inhibitors in PKG-expressing cells and enhanced by overexpression of PKG; the effect should be absent in PKG-deficient cells and restored by transfection of PKG. The PKG inhibitor KT5823 cannot be used as a sole criterion, because its specificity and effectiveness in intact cells has been seriously questioned.<sup>14</sup>

We will discuss specific genes regulated by cGMP, and review mechanisms of transcriptional and posttranscriptional regulation by cGMP.

### (Patho)Physiological Processes Involving cGMP Regulation of Gene Expression

At the present time, cGMP-mediated increases or decreases in mRNA expression fulfilling the minimal criteria described above have been reported for >50 different genes, but in most cases the mechanisms of cGMP regulation have not been explored. cDNA microarray analyses of gene expression in rat aorta or cardiomyocytes after exposure to NO donors have revealed hundreds of genes changed by NO.<sup>15,16</sup> Similarly, hearts of eNOS-, nNOS-, and ANP-deficient mice have been compared with hearts of wild-type mice and microarray analyses have demonstrated divergent transcriptional programs, some of which may be related to alterations in cGMP signaling.<sup>17,18</sup> Although microarray analyses are providing large amounts of information regarding changes in gene expression in response to specific signals or gene alterations,

only detailed analysis of single genes is able to distinguish direct versus indirect effects and determine the physiological significance of these changes. Table 1 summarizes cGMP regulation of specific genes that are discussed in the following sections.

### Regulation of Vascular Tone

NO regulates vascular tone through stimulation of sGC, and ANP/BNP through stimulation of rGC-A, with cGMP activation of PKG causing smooth muscle relaxation via multiple mechanisms, including lowering of intracellular  $\text{Ca}^{2+}$  and inhibition of RhoA-dependent  $\text{Ca}^{2+}$  sensitization of contraction.<sup>3,19</sup> However, cGMP also positively regulates RhoA expression in vascular smooth muscle cells (VSMCs).<sup>20</sup> Prolonged exposure to nitrates leads to downregulation of sGC and PKG expression associated with nitrate tolerance,<sup>21,22</sup> and prolonged elevation of circulating ANP leads to downregulation of rGC.<sup>23</sup> These negative feedback loops involve cGMP inhibition of sGC, PKG, and rGC expression.

### Soluble Guanylate Cyclase (sGC)

Low concentrations of NO donors, ANP, or cGMP analogues decrease mRNA and protein levels of both sGC- $\alpha_1$  and - $\beta_1$  subunits in VSMCs by >90% within 24 hours.<sup>21</sup> cGMP accelerates the decay of sGC- $\alpha_1$  and  $\beta_1$  mRNA; the effect is mediated by cGMP-induced downregulation of the RNA-stabilizing protein HuR (described later).<sup>21,24</sup>

### PKG

Continuous exposure to NO-releasing agents, cGMP, or cAMP analogues suppresses PKG mRNA and protein levels in VSMCs by decreasing transcription without affecting mRNA stability.<sup>22</sup> Similarly, reduced PKG levels are found in cGMP-treated cardiomyocytes, and in the aortas of transgenic mice overexpressing eNOS in the endothelium.<sup>6,25</sup> Two SP-1 binding sites appear to be required for basal PKG promoter activity, and SP-1 and SP-3 DNA binding activities are reduced in cyclic nucleotide-treated cells.<sup>26</sup>

### Receptor Guanylate Cyclase A (rGC-A)

Ligand-dependent downregulation of rGC-A is associated with cGMP-mediated reduction in rGC-A mRNA levels and promoter activity in VSMCs.<sup>23</sup> Correspondingly, rGC-A expression is upregulated in ANP-deficient mice.<sup>27</sup> The effect of cGMP on the rGC-A promoter is not mimicked by cAMP; it is mediated by a negative *cis*-acting element localized approximately 1400 nucleotides upstream of the transcription start site, but the *trans*-acting factors remain to be identified.<sup>23</sup>

### RhoA

Long-term exposure of primary VSMCs to NO donors or cGMP analogues increases RhoA expression through increased *rhoA* transcription as well increased RhoA protein stability due to PKG phosphorylation.<sup>20</sup> The PKG-mediated transcriptional effect requires an intact CRE in the RhoA promoter and is associated with increased phosphorylation of CREB and ATF-1.<sup>20</sup> Inhibition of NO synthesis in rats decreases RhoA mRNA and protein expression in aorta and pulmonary artery, suggesting that basal release of NO is

**TABLE 1. Regulation of Specific Genes by cGMP**

| Gene                                          | Cell Type                | Culture Conditions   | Regulation by cGMP           | PKG-Mediated* | Reference     |
|-----------------------------------------------|--------------------------|----------------------|------------------------------|---------------|---------------|
| Genes involved in regulation of vascular tone |                          |                      |                              |               |               |
| sGC ( $\alpha_1$ , $\beta_1$ )                | VSMCs                    |                      | ↓ mRNA stability (HuR)       | ND            | 21, 24        |
| PKG                                           | VSMCs                    |                      | ↓ mRNA, ↓ transcription      | ND            | 6, 22, 25, 26 |
| rGC-A                                         | VSMCs, RTCs              |                      | ↓ mRNA, ↓ transcription      | ND            | 23, 27        |
| RhoA                                          | VSMCs                    |                      | ↑ mRNA, ↑ transcription      | Yes           | 20            |
| Genes associated with cardiac hypertrophy     |                          |                      |                              |               |               |
| ANP                                           | CMs                      | PE-stimulated        | ↓ mRNA                       | Yes           | 6             |
| BNP                                           | CMs                      | PE-stimulated        | ↓ mRNA, ↓ transcription      | Yes           | 30            |
| Skeletal $\alpha$ -actin                      | CMs                      | PE-stimulated        | ↓ mRNA                       | ND            | 29            |
| $\beta$ -MHC                                  | CMs                      | PE-stimulated        | ↓ mRNA                       | ND            | 29            |
| MLP                                           | CMs                      | ET-1-stimulated      | ↓ mRNA                       | Yes           | 16            |
| Genes associated with VSMC differentiation    |                          |                      |                              |               |               |
| SM-MHC-2                                      | VSMCs                    | Dedifferentiated     | ↑ protein, ↑ transcription?  | Yes           | 32, 35–37     |
| SM- $\alpha$ -actin                           | VSMCs                    | transfected with PKG | ↑ protein, ↑ transcription?  | Yes           | 32            |
| SM-calponin                                   | VSMCs                    |                      | ↑ protein, ↑ transcription?  | Yes           | 32            |
| Osteopontin                                   | VSMCs                    |                      | ↓ protein, ↓ translation?    | Yes           | 33            |
| Thrombospondin                                | VSMCs                    |                      | ↓ protein, ↓ translation?    | Yes           | 33            |
| FGFR-1/2                                      | VSMCs                    |                      | ↓ mRNA                       | Yes           | 34            |
| Genes associated with proliferation           |                          |                      |                              |               |               |
| MKP-1                                         | VSMCs, ECs, MCs, BHK     |                      | ↑ mRNA                       | Yes           | 53, 59–61     |
| Cyclins A, D1, E                              | VSMCs, MCs               |                      | ↓ mRNA                       | ND            | 48, 50, 62    |
| p21 <sup>Waf1/Cip1</sup>                      | Fibroblasts              |                      | ↑ mRNA                       | ND            | 36, 49        |
| p16 <sup>INK4a</sup>                          | VSMCs                    |                      | ↑ mRNA                       | ND            | 36            |
| ET-1                                          | ECs, Cardiac fibroblasts |                      | ↓ mRNA                       | ND            | 52, 55        |
| VEGF                                          | VSMCs, other             | Normoxic             | ↑ mRNA                       | ND            | 63, 64        |
|                                               | VSMCs, ECs               | Hypoxic              | ↓ mRNA, ↓ transcription      | ND            | 65            |
| Genes associated with apoptosis               |                          |                      |                              |               |               |
| Mcl-1                                         | CMs                      |                      | ↓ mRNA                       | ND            | 67            |
| Bcl-2                                         | Neuronal cells           |                      | ↑ mRNA                       | Yes           | 73            |
|                                               | B lymphocytes            |                      | ↑ mRNA                       | ND            | 75            |
| Tpx-1                                         | Neuronal cells           |                      | ↑ mRNA                       | Yes           | 9             |
| BNIP3                                         | Hepatocytes              |                      | ↓ mRNA                       | ND            | 74            |
| Other genes                                   |                          |                      |                              |               |               |
| PGC-1                                         | 3T3-L1, U937, HeLa       |                      | ↑ mRNA                       | ND            | 76            |
| TNF- $\alpha$                                 | CMs, VSMCs               | Unstimulated         | ↑ mRNA                       | Yes           | 77, 84        |
|                                               | Macrophages, glia        | Unstimulated         | ↑ mRNA                       | ND            | 85            |
|                                               | Macrophages              | LPS-stimulated       | ↓ mRNA stability             | ND            | 86            |
| NOS-2                                         | CMs, VSMCs               | Cytokine-stimulated  | ↑ mRNA                       | Yes           | 79, 84        |
|                                               | MCs                      | Cytokine-stimulated  | ↑ mRNA (<12 hours)           | ND            | 78, 81        |
|                                               | MCs                      | Cytokine-stimulated  | ↓ mRNA stability (>24 hours) |               |               |
|                                               | Macrophages              | LPS-stimulated       | ↓ mRNA stability             | ND            | 87            |
| COX-2                                         | RTCs                     | Salt-depleted        | ↑ mRNA                       | ND            | 89            |
|                                               | MCs                      | Cytokine-stimulated  | ↑ transcription (<8 hours)   | ND            | 82, 88        |
|                                               | MCs                      | Cytokine-stimulated  | ↓ mRNA stability (>24 hours) |               |               |
|                                               | Macrophages              | LPS-stimulated       | ↓ mRNA stability             | ND            | 87            |
| PAI-1                                         | VSMCs, ECs               | Ang II-stimulated    | ↓ mRNA                       | ND            | 90, 91        |
| MMP-13                                        | ECs                      |                      | ↑ mRNA, ↑ transcription      | ND            | 131           |
| HO-1                                          | ECs                      | Serum-starved        | ↑ mRNA, ↑ transcription      | No            | 132           |
| TGF- $\beta_3$                                | Cardiac fibroblasts      |                      | ↓ mRNA stability             | ND            | 140           |
| KCl cotransporter-3                           | VSMCs                    |                      | ↑ mRNA stability             | ND            | 142           |

Ang II indicates angiotensin II; CMs, cardiomyocytes; ECs, endothelial cells; ET-1, endothelin-1; FGFR, fibroblast growth factor receptor; HO-1, heme oxygenase-1; HuR, Hu family RNA-binding protein; KCl cotransporter-3, potassium chloride cotransporter-3; LPS, lipopolysaccharide; MCs, mesangial cells; MHC, myosin heavy chain; MKP-1, MAP kinase phosphatase-1; MLP, muscle LIM protein; MMP-13, matrix metalloproteinase-13; PAI-1, plasminogen activator inhibitor-1; PE, phenylephrine; PGC-1, peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$ ; RTCs, renal tubule cells; TGF- $\beta_3$ , transforming growth factor- $\beta_3$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; and VEGF, vascular endothelial growth factor.

\*ND indicates not determined; yes, effect observed in PKG-expressing cells and inhibited by specific cell-permeable PKG inhibitor and/or enhanced by PKG overexpression.

necessary to maintain RhoA expression and function in VSMCs.<sup>20</sup>

### Cardiac Hypertrophy

Cardiac hypertrophy involves induction of embryonic genes, including ANP, BNP, and skeletal  $\alpha$ -actin, by hypertrophic stimuli such as ET-1, angiotensin II (Ang II), phenylephrine, and fibroblast growth factor (FGF).<sup>28</sup> Hypertrophy-promoting signaling pathways include the small GTPases Ras, RhoA, and Rac, mitogen-activated protein kinases (MAP kinases), and  $\text{Ca}^{2+}$ /calcineurin.<sup>28</sup> Mice deficient in ANP or rGC-A develop increased ventricular mass out of proportion to the mild changes in blood pressure,<sup>2</sup> and mice deficient in nNOS develop cardiac hypertrophy with normal systemic blood pressure,<sup>11</sup> suggesting that NO/cGMP negatively regulate cardiac hypertrophy. PKG-deficient mice may not live long enough to develop cardiac hypertrophy, but PKG overexpression enhances the antihypertrophic effects of NO in phenylephrine-stimulated cardiomyocytes in vitro, and prevents phenylephrine-induced increase in prepro-ANP mRNA.<sup>6</sup> Similarly, natriuretic peptides and cGMP analogues suppress the phenylephrine-induced hypertrophic pattern of gene expression including skeletal  $\alpha$ -actin and  $\beta$ -myosin heavy chain gene expression.<sup>29</sup> Because RhoA signaling is required for phenylephrine-induced hypertrophic gene expression, and cardiac-specific overexpression of serum response factor (SRF) results in hypertrophy, it is possible that some of PKGs antihypertrophic effects could be through inhibition of RhoA signaling to SRF.<sup>5,28</sup> NO/cGMP activation of PKG also inhibits  $\text{Ca}^{2+}$ -dependent activation of the hypertrophic calcineurin-NF/AT pathway, preventing NF/AT induction of BNP mRNA and promoter activity.<sup>30</sup> cGMP inhibition of SRF- and NF/AT-dependent transcription is further discussed below. Cardiac expression profiling of genes stimulated by the hypertrophic peptide ET-1 in the presence or absence of NO demonstrates downregulation of muscle LIM protein (MLP) by NO; this effect is at least partially through cGMP/PKG, and may explain some of PKGs antihypertrophic effects, because MLP expression is necessary and sufficient for ET-1-induced hypertrophy.<sup>16</sup> In addition, ET-1 synthesis is negatively regulated by cGMP (see later).

### VSMC Differentiation/Phenotypic Modulation

In response to vascular injury and during in vitro culture, VSMCs change their state of differentiation from a highly differentiated, "contractile" phenotype to a dedifferentiated "synthetic" phenotype; during this process, they acquire the capacity to proliferate, migrate, and produce extracellular matrix proteins such as osteopontin and thrombospondin, thus contributing to neointima formation after vascular injury.<sup>31</sup> Dedifferentiation to the synthetic phenotype is associated with loss of PKG expression and transcriptional downregulation of contractile proteins such as smooth muscle myosin heavy chain-2 (SM-MHC-2), SM- $\alpha$ -actin, and SM calponin.<sup>32</sup>

Transfecting synthetic VSMCs with constitutively active or wild-type PKG (plus cGMP stimulation) restores a more contractile phenotype with fusiform morphology, increased expression of SM-MHC-2, SM- $\alpha$ -actin, and calponin protein,

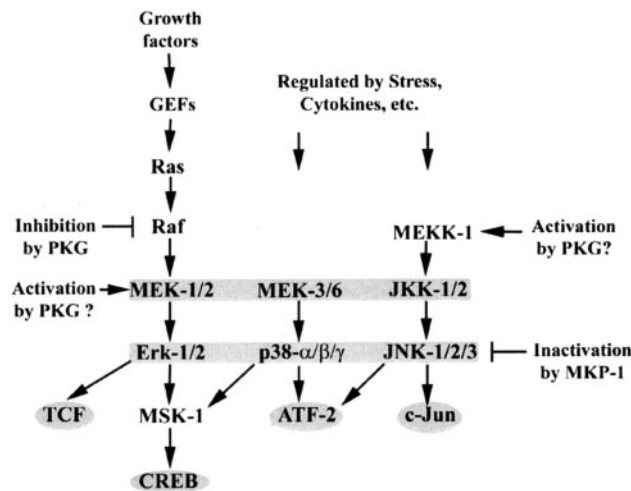
and decreased expression of osteopontin, thrombospondin, and FGF receptors-1/2.<sup>31–34</sup> FGF receptor protein and mRNA levels are dramatically and proportionally decreased in PKG-transfected cells compared with control-transfected cells, but osteopontin and thrombospondin mRNA levels decrease only minimally, suggesting PKG-induced changes in mRNA translation or protein stability.<sup>33,34</sup> Whether the PKG-mediated induction of contractile protein expression occurs at the transcriptional level is unclear, although one preliminary report suggests that PKG activation may activate the SM-MHC promoter.<sup>35</sup> In addition, SM-MHC-2 mRNA and protein expression is increased in CNP peptide-overexpressing VSMCs in vitro and in vivo after angioplasty,<sup>36</sup> and downregulation of the SM-MHC promoter by platelet-derived growth factor is prevented by NO.<sup>37</sup> These data suggest that PKG plays an important role during VSMC phenotypic modulation, positively and negatively regulating gene expression. A preliminary report of cDNA microarray analyses comparing PKG-transfected and control-transfected, late passage VSMCs suggests that >100 transcripts may be up- or downregulated more than 3-fold by cGMP/PKG.<sup>38</sup>

The mechanism(s) responsible for PKG downregulation in dedifferentiating VSMCs remains unknown, but PKG mRNA is downregulated when differentiated VSMCs are chronically exposed to NO, cyclic nucleotides, or various growth factors in vitro.<sup>22,31</sup> PKG levels may transiently decrease after vascular injury in vivo, and appear to decrease in proliferating neointimal VSMCs coincidentally with transcriptional downregulation of contractile marker expression and increased synthesis of osteopontin.<sup>39–42</sup> Increased growth factor and cytokine production at the site of vascular injury could increase local cGMP through iNOS induction and may explain PKG downregulation.<sup>31</sup> Reexpression of constitutively active PKG (or wild type PKG with cGMP stimulation) inhibits VSMC migration, enhances apoptosis, reduces proliferation, and decreases neointima formation after vascular injury.<sup>32,39</sup> These results suggest that PKG modulates the VSMC phenotype in vivo and are consistent with the finding that adenoviral overexpression of CNP or soluble guanylate cyclase reduces neointima formation, whereas eNOS-deficient mice demonstrate a hyperplastic response after vascular injury.<sup>36,43,44</sup>

### Regulation of Cell Proliferation

Depending on the cell type, cGMP can have pro- or antiproliferative effects. In VSMCs, mesangial cells, and various fibroblasts, cGMP inhibits proliferation, and the effect is mostly mediated by PKG, although it may involve PKA cross-activation under some conditions.<sup>4,45–54</sup> The antiproliferative effect correlates with cGMP inhibition of growth factor-induced extracellular signal-regulated kinase (Erk-1/2) activity (online Table, available in the online data supplement at <http://www.circresaha.org>; also discussed later), increased expression of MAP kinase phosphatase-1 (MKP-1),<sup>46,47,53</sup> modulation of cell cycle-associated genes, and reduction of ET-1 synthesis.<sup>52,55</sup>

In contrast to the antiproliferative effects in VSMCs and fibroblasts, cGMP increases proliferation of endothelial cells.<sup>36,56–58</sup> The proproliferative effect of cGMP in endothe-



**Figure 2.** Regulation of MAP kinase pathways by cGMP. Three MAP kinases comprise the extracellular signal-regulated kinases Erk-1/2, p38 isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$ , and the c-Jun amino terminal kinases JNK-1/2/3. Upstream kinases include the MAP kinase kinases MEK-1/2 and MEK-3/6 for Erk and p38, respectively, and the JNK kinases JKK-1/2 for JNK-1/2/3. All three pathways are activated by multiple extracellular stimuli, including growth factors, cytokines, and stress (such as oxidative stress and heat shock), and all three types of MAP kinases are inactivated by MAP kinase phosphatase-1 (MKP-1). Some transcription factors targeted by MAP kinases are shown; a more complete list has been reviewed.<sup>92</sup> cGMP can either activate or inhibit each pathway, depending on the cell type and growth conditions (online Table). Inhibition of Raf-1 by cGMP/PKG<sup>53</sup> and PKG-dependent activation of MEK-1/2,<sup>106,133</sup> MEK-3,<sup>139</sup> or MEKK-1<sup>71</sup> have been reported; however, it is not clear whether PKG directly activates these enzymes (indicated by “?”). GEFs, guanine nucleotide exchange factors.

lial cells correlates with increased Erk-1/2 activity, and may be related to increased production of vascular endothelial growth factor (VEGF).<sup>56–58</sup>

#### MAP Kinase Phosphatase-1 (MKP-1)

MKP-1 dephosphorylates and inactivates the MAP kinases Erk-1/2, p38, and c-Jun N-terminal kinase (JNK) (Figure 2).<sup>59</sup> MKP-1 mRNA expression is increased by NO-releasing agents, ANP, and cGMP analogues in endothelial and smooth muscle cells<sup>59–61</sup>; induction by cGMP can occur in the absence of Erk-1/2 stimulation.<sup>53</sup> cGMP, via PKG, induces MKP-1 mRNA sufficiently to inhibit growth factor-induced Erk-1/2 activity, and MKP-1 induction contributes to the antiproliferative effect of cGMP-elevating agents.<sup>53,60</sup>

#### Cyclins and Cyclin Inhibitors

cGMP decreases expression of cell cycle-promoting genes, such as cyclin A, D1, and E.<sup>48,50,62</sup> In addition, cGMP may increase expression of cell cycle inhibitors, such as p21<sup>Waf1/Cip1</sup> and p16<sup>INK4a</sup>.<sup>36,49</sup> Under some conditions, cGMP-induced changes in expression of cell cycle-associated genes appear to be dependent on inhibition of Erk-1/2, but this is not always the case.<sup>47,49,50</sup>

#### Endothelin-1 (ET-1)

ET-1 is a potent vasoconstrictor and mitogenic peptide produced by proteolytic cleavage from an inactive precursor and secreted by endothelial cells.<sup>55</sup> Basal prepro-ET-1 mRNA

levels and peptide synthesis appear to be decreased by cGMP, because incubation of endothelial cells with NOS inhibitors, NO scavenger molecules, or sGC inhibitors increase prepro-ET-1 synthesis and ET-1 peptide secretion, and this effect is reversed by cGMP analogues.<sup>55</sup> Thrombin- or Ang II-mediated increases in prepro-ET-1 mRNA levels are also inhibited by natriuretic peptides and cGMP analogues.<sup>52</sup>

#### Vascular Endothelial Growth Factor (VEGF)

VEGF mediates angiogenesis and vascular permeability; in normoxic VSMCs, hepatoma, and glioma cells, NO donors increase basal VEGF mRNA levels in a cGMP-dependent fashion.<sup>63,64</sup> An in vivo model of embolic stroke suggests a positive role for cGMP in angiogenesis, which is dependent on VEGF.<sup>56</sup> However, VEGF expression is induced by hypoxia, and treating hypoxic VSMCs or endothelial cells with NO or cGMP decreases VEGF mRNA,<sup>65</sup> and NOS inhibition increases VEGF mRNA in balloon-injured arterial walls, suggesting that endogenous NO may suppress VEGF expression under these conditions.<sup>66</sup>

#### Apoptosis

Pro- and antiapoptotic effects of cGMP have been described in different cell types. In VSMCs, cardiomyocytes, and endothelial cells, NO, natriuretic peptides, and cGMP analogues increase apoptosis, and the effect appears to be PKG mediated.<sup>45,67–69</sup> In cardiomyocytes, cGMP sharply decreases mRNA expression of the antiapoptotic Bcl-2 homologue Mcl-1.<sup>67</sup> In VSMCs and endothelial cells, the effect of cGMP occurs in low serum-containing medium and is counteracted by Ang II and ET-1, respectively.<sup>68,69</sup> The mechanism(s) of the proapoptotic effects of cGMP in vascular cells remains to be determined, but cGMP may increase apoptosis through PKG-dependent activation of JNK and/or phosphorylation and inactivation of  $\beta$ -catenin.<sup>70–72</sup>

In neuronal cells, hepatocytes, and lymphocytes, cGMP has antiapoptotic effects that appear to be mediated by Bcl-2 or Bcl-2-associated proteins.<sup>73–75</sup> In neuronal cells, prolonged inhibition of NOS or sGC induces apoptosis that is prevented by cGMP analogues; the protective effect of cGMP is associated with increased CREB phosphorylation and increased mRNA and protein expression of the apoptosis inhibitor Bcl-2.<sup>73</sup> During growth factor deprivation, cGMP/PKG protect neuronal cells from apoptosis by increasing expression of the oxidative stress-related proteins thioredoxin and thioredoxin peroxidase (Tpx-1), which leads to increased Bcl-2 expression.<sup>9</sup>

#### Mitochondrial Biogenesis

NO, in a cGMP-dependent fashion, triggers mitochondrial biogenesis in many different cell types, and mitochondrial biogenesis induced by cold exposure is reduced in eNOS-deficient mice.<sup>76</sup> The cGMP effect is mediated by induction of peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1), a master regulatory factor of mitochondrial biogenesis in brown adipose tissue and in cardiac and skeletal muscle; this coactivator increases expression of other transcription factors that regulate nuclear and mitochondrial genes encoding mitochondrial proteins.<sup>76</sup>

### Other cGMP-Regulated Genes

cGMP regulates expression of the inflammatory cytokine TNF- $\alpha$ , and the cytokine-inducible genes iNOS and COX-2 involved in inflammatory processes.<sup>77–80</sup> Both positive and negative effects of cGMP have been described for each of these genes, in some cases leading to biphasic regulation, eg, early transcriptional upregulation followed by downregulation due to mRNA destabilization; the latter could be related to HuR downregulation by cGMP (discussed later).<sup>81–83</sup>

#### *Tumor Necrosis Factor- $\alpha$ (TNF- $\alpha$ )*

In cardiomyocytes, NO increases TNF- $\alpha$  mRNA in a cGMP- and PKG-dependent fashion, and this effect correlates with increased nuclear factor- $\kappa$ B (NF- $\kappa$ B) activity and is mimicked by cGMP, but not cAMP analogues.<sup>77</sup> In VSMCs and resting macrophages, cGMP analogues induce basal TNF- $\alpha$  mRNA and enhance IL-1-induced TNF- $\alpha$  mRNA.<sup>84,85</sup> However, in LPS-stimulated macrophages, induction of TNF- $\alpha$  mRNA and protein is attenuated by cGMP due to mRNA destabilization.<sup>86</sup>

#### *Inducible NO Synthase (iNOS)*

In cardiomyocytes, VSMCs, and mesangial cells, cytokine-induced transcription of iNOS is enhanced by NO donors, ANP/BNP, or cGMP analogues, and reduced by NOS inhibitors, whereas in the absence of cytokines, cGMP-elevating agents have very little or no effect on basal iNOS expression.<sup>78,79,81,84</sup> In addition, in some cell types, cGMP destabilizes iNOS mRNA leading to downregulation of cytokine-induced iNOS mRNA at later time points.<sup>81,87</sup>

#### *Inducible Cyclooxygenase-2 (COX-2)*

Like iNOS, COX-2 is transcriptionally induced in response to inflammatory stimuli, and the effects of NO on cytokine-induced COX-2 and iNOS expression are similar.<sup>80</sup> NO is necessary for cytokine induction of COX-2 mRNA in mesangial cells, and NO donors, ANP, and cGMP analogues synergistically enhance the effect of IL-1 $\beta$  on COX-2 mRNA at early time points.<sup>82,88</sup> However, at later time points, NO donors decrease cytokine-induced COX-2 mRNA levels, reminiscent of the biphasic effects of NO on iNOS expression in these cells.<sup>81,82</sup> Destabilization of COX-2 mRNA by ANP and cGMP analogues has been described in LPS-stimulated macrophages.<sup>87</sup>

In whole animal models, NO positively affects COX-2 expression, and its importance for prostaglandin synthesis has been confirmed in iNOS-deficient mice.<sup>80</sup> Induction of COX-2 mRNA by salt depletion and/or angiotensin-converting enzyme inhibition in renal cortex is blocked by NOS inhibitors, and this effect is relieved by cGMP analogues; correspondingly, NO donors and cGMP analogues increase basal COX-2 mRNA levels in renal tubule cells.<sup>89</sup>

#### *Plasminogen Activator Inhibitor-1 (PAI-1)*

PAI-1 is produced by VSMCs and endothelial cells, and elevated levels are found in atherosclerotic and balloon-injured vessels.<sup>90</sup> NO donors, natriuretic peptides, and cGMP analogues reduce Ang II-induced PAI-1 mRNA levels without affecting mRNA stability.<sup>90,91</sup>

## Mechanisms of Transcriptional Regulation by cGMP

### Overview

cGMP can regulate transcription factors directly by inducing phosphorylation or by increasing expression of short-lived proteins. Transcription factors controlled by cGMP-dependent phosphorylation include the cAMP response-element (CRE)-binding protein CREB, activating transcription factor-1 (ATF-1), and the multifunctional transcription factor TFII-I (summarized in Table 2). Transcription factors whose expression is regulated by cGMP include the AP-1 family proteins c-Fos and JunB, the early growth response gene Egr-1, and the growth arrest-specific homeobox gene GAX (Table 2). In addition, cGMP can regulate transcription factors indirectly, through modulation of upstream signal transduction pathways. Examples include cGMP regulation of an inhibitor of NF- $\kappa$ B, and inhibition of calcineurin signaling to NF/AT and of RhoA signaling to SRF (Table 2). Through activation or inhibition of MAP kinase pathways, cGMP can regulate the activity of multiple transcription factors, including ternary complex factor (TCF), CREB, ATF-2, and c-Jun (online Table and Figure 2).<sup>92</sup>

### Transcription Factors Directly Controlled by cGMP-Dependent Phosphorylation

#### *CREB and ATF-1*

The transcription factor CREB is activated by a diverse array of stimuli through phosphorylation of Ser<sup>133</sup> in its kinase-inducible domain; CREB is critical for proliferation, differentiation, and survival of many different cell types, and absence of CREB causes dwarfism and cardiac myopathy.<sup>93</sup> Increased intracellular cGMP leads to increased CREB Ser<sup>133</sup> phosphorylation in VSMCs, neuronal cells, and PKG-transfected Baby Hamster Kidney (BHK) cells, but not in PKG-deficient BHK cells.<sup>20,73,94,95</sup> cGMP-induced CREB phosphorylation in BHK cells occurs at physiologically relevant PKG levels and is independent of changes in intracellular Ca<sup>2+</sup>, activation of MAP kinases (Erk-1/2 and p38), or cross-activation of PKA, thus ruling out the effect of other known CREB kinases.<sup>95</sup> PKG can directly phosphorylate CREB on Ser<sup>133</sup> in vitro; the kinetics are slower than those of PKA, but comparable to those of Ca<sup>2+</sup>/calmodulin-dependent protein kinase IV.<sup>95</sup> Correspondingly, PKG is less effective than PKA, but similar to Ca<sup>2+</sup>/calmodulin-dependent protein kinase, in stimulating cAMP-response-element (CRE)-dependent transcription in intact cells.<sup>95–98</sup> In BHK cells and in some neuronal cells, cGMP-mediated transactivation of the *c-fos* promoter is dependent on CREB phosphorylation and nuclear translocation of PKG, as demonstrated by the ineffectiveness of extranuclear PKG constructs.<sup>95,99</sup> Nuclear translocation of PKG has been demonstrated in neuronal cells, neutrophils, macrophages, and some embryonal smooth muscle cells.<sup>95,99–102</sup> However, other investigators found no evidence of PKG nuclear translocation in primary VSMCs, HEK293, and CV-1 cells, or observed nuclear PKG only in a minority of the cell population.<sup>97,103,104</sup> Based on recent findings, we have speculated that PKG may be retained in extranuclear compartments by binding to cell

**TABLE 2. Transcription Factors Regulated by cGMP**

| Transcription Factor | Cell Type                                                  | Mechanism of Regulation                                                               | Effect of cGMP                                         | References     |
|----------------------|------------------------------------------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------|----------------|
| CREB                 | Neuronal cells                                             | ↑ phosphorylation of CREB Ser <sup>133</sup><br>(directly or indirectly PKG-mediated) | ↑ transcriptional activity                             | 73, 94         |
|                      | VSMCs                                                      |                                                                                       |                                                        | 20             |
|                      | BHK*                                                       |                                                                                       |                                                        | 95             |
| ATF-1                | VSMCs                                                      | ↑ ATF-1 phosphorylation<br>(PKG-dependent)                                            | ↑ transcriptional activity?                            | 20             |
|                      | BHK*                                                       |                                                                                       |                                                        | 95             |
| TFII-I               | COS-7,* BHK,* C2C12 myoblasts                              | Direct TFII-I phosphorylation by<br>PKG/association with PKG I $\beta$                | ↑ transcriptional activity                             | 105            |
| AP-1 (c-Fos, Jun B)  | Neuronal cells, REF52, thyroid<br>cells, BHK,* other cells | ↑ transcription (CREB-dependent)                                                      | ↑ mRNA                                                 | 110, 112       |
|                      |                                                            |                                                                                       | ↑ AP-1 DNA binding                                     |                |
|                      |                                                            |                                                                                       | ↑ transcriptional activity<br>(in serum-starved cells) | 111<br>96, 113 |
|                      | Cardiac fibroblasts                                        |                                                                                       | ↑ Ang II-stimulated <i>c-fos</i> mRNA                  | 51             |
|                      | Endothelial cells                                          |                                                                                       | ↑ TNF- $\alpha$ -stimulated AP-1 DNA binding           | 114            |
| Egr-1                | Neuronal cells                                             | ↑ transcription                                                                       | ↑ mRNA, ↑ DNA binding,<br>↑ transcriptional activity   | 8, 110, 119    |
| GAX                  | VSMCs                                                      | ?                                                                                     | ↑ mRNA                                                 | 120            |
| NF- $\kappa$ B       | T lymphocytes, other cells                                 | p50/p65 phosphorylation                                                               | ↑ DNA binding/transcriptional activity                 | 122            |
|                      | Cardiomyocytes                                             | I $\kappa$ B phosphorylation/destabilization                                          | ↑ DNA binding/transcriptional activity                 | 77             |
|                      | Endothelial cells, hepatocytes                             | I $\kappa$ B stabilization                                                            | ↓ cytokine-induced DNA binding of NF- $\kappa$ B       | 123, 124, 125  |
| NF/AT                | Cardiomyocytes                                             | ↓ calcium influx/<br>↓ calcineurin activity                                           | ↓ NF/AT nuclear translocation                          | 30             |
|                      |                                                            |                                                                                       | ↓ transcriptional activity                             |                |
| SRF                  | VSMCs, cardiomyocytes                                      | ↓ Rho-dependent activation#                                                           | ↓ SRF-dependent transcription#                         | 5              |

\*Transfection of PKG required for the effect of cGMP in PKG-deficient cells; #SRF/TCF complexes on the *fos* and *egr-1* promoters are insensitive to Rho signaling.<sup>128</sup>

type-specific anchoring proteins.<sup>105</sup> In primary VSMCs, cGMP/PKG-induced CREB phosphorylation may be indirect, eg, via activation of MAP kinases (Figure 2 and see later discussion).<sup>20,106</sup> The closely related CREB family member ATF-1 is also phosphorylated in response to PKG activation by cGMP, and ATF-1 appears to mediate transcriptional activation of the RhoA promoter by PKG.<sup>20,95</sup>

### TFII-I

TFII-I is a transcriptional regulator for many genes, including *c-fos* and so-called endoplasmic reticulum stress-response genes; TFII-I interacts with multiple transcription factors including SRF, TCF, ATF-6, c-Myc, and NF- $\kappa$ B, and with histone deacetylases.<sup>107–109</sup> We found that TFII-I physically interacts with PKG I $\beta$ , with these two proteins coimmunoprecipitating in C2C12 myoblasts.<sup>105</sup> PKG phosphorylates TFII-I in vitro and in vivo; PKG I $\beta$  activation enhances the transactivation potential of wild type, but not phosphorylation-deficient mutant TFII-I on an SRF/TCF-dependent reporter gene, and PKG cooperates with TFII-I to transactivate the *fos* promoter.<sup>105</sup>

### Short-Lived Transcription Factors With Expression Regulated by cGMP

#### AP-1 (*Fos/Jun*)

Fos and Jun proteins dimerize to form the AP-1 transcription factor complex and are important for cell cycle progression and apoptosis; resting cells typically express low levels of *fos* and *jun* mRNA, but transcription of AP-1-related genes is rapidly induced in response to many stimuli.<sup>72</sup> NO-releasing

agents, natriuretic peptides, and cGMP analogues increase *c-fos* and *junB* mRNA expression in cultured cells and primary tissues,<sup>110–112</sup> and NOS inhibitors can reduce *c-fos* expression.<sup>112</sup> Cardiac expression profiling from eNOS-deficient mice demonstrate decreased *c-fos* mRNA expression compared with wild-type mice.<sup>17</sup> In cardiac fibroblasts, cGMP-elevating agents modestly enhance *c-fos* induction by Ang II.<sup>51</sup> As a consequence of NO/cGMP-induced *c-fos* and *junB* mRNA expression, AP-1 DNA binding activity increases resulting in increased transcription of AP-1-dependent reporter genes and endogenous genes.<sup>110,111,113</sup> In pulmonary endothelial cells, NO-releasing agents and cGMP analogues increase AP-1 DNA binding activity only if cells are costimulated with TNF- $\alpha$ .<sup>114</sup>

Although high concentrations of NO can decrease DNA binding of recombinant Fos and Jun in vitro through S-nitrosylation and/or S-glutathionylation, the in vivo significance of this effect is unknown.<sup>115,116</sup> We demonstrated that NO stimulation of the *fos* promoter in different cell types is strictly cGMP-dependent and requires PKG activity.<sup>95,117</sup> PKG targets several transcriptional elements in the *fos* promoter: the CRE and the *fos* AP-1 site, which both bind CREB-related proteins, and the serum response element (SRE), which binds multiple transcription factors including SRF, TCF, C/EBP- $\beta$ , and TFII-I.<sup>95,96,105</sup> cGMP/PKG transactivation of the *fos* promoter requires CREB-related proteins, but is independent of AP-1 and C/EBP-related transcription factors and can occur independently of MAP kinase activation by cGMP.<sup>95</sup>

**Egr-1**

Transcription of *egr-1* is induced by many growth factors and stress stimuli with similar kinetics as *c-fos*.<sup>118</sup> Egr-1 plays an important role in cell growth, differentiation, and apoptosis, and high, sustained expression of Egr-1 has been observed in atherosclerotic lesions.<sup>118</sup> In pheochromocytoma cells, NO-releasing agents and natriuretic peptides increase *egr-1* promoter activity, mRNA, and protein expression, and increase transcription from an Egr-1-responsive reporter.<sup>110,119</sup> Moreover, cGMP analogues increase serum-stimulated Egr-1 DNA binding activity; this effect is enhanced by overexpression of PKG and occurs in the absence of significant changes in MAP kinase activity.<sup>8</sup> In contrast, NO and cGMP analogues appear to have no effect on Ang II-induced *egr-1* mRNA expression in cardiac fibroblasts.<sup>51</sup>

**The Growth Arrest-Specific Homeobox Gene (GAX)**

GAX is found predominantly in cardiovascular tissues; it is expressed in quiescent VSMCs and rapidly downregulated after mitogen-stimulation or after vascular injury; overexpression of GAX induces growth arrest.<sup>118,120</sup> CNP and cGMP analogues increase GAX mRNA expression in serum-starved primary VSMCs and largely prevent the decrease in GAX mRNA in Ang II-stimulated cells;<sup>120</sup> this could be important for VSMC phenotypic modulation.<sup>118</sup>

**Transcription Factors Indirectly Regulated by cGMP****NF- $\kappa$ B**

NF- $\kappa$ B is a transcriptional activator of genes involved in inflammation, including cytokines and adhesion molecules.<sup>121</sup> NF- $\kappa$ B subunits are inactive when bound to cytoplasmic I $\kappa$ B proteins, but multiple stimuli can induce phosphorylation and degradation of I $\kappa$ B, thereby allowing activation and nuclear translocation of NF- $\kappa$ B.<sup>121</sup> NO can increase or decrease NF- $\kappa$ B activity depending on the NO concentration, redox milieu, cell type, and co-stimulus, and in most cases, the effects of high NO concentrations on NF- $\kappa$ B appear to be cGMP-independent.<sup>12,13,80</sup> However, in cardiomyocytes, NO-donors increase NF- $\kappa$ B (p50/p65) DNA binding activity and induce expression of an NF- $\kappa$ B-responsive gene in a cGMP/PKG-dependent fashion.<sup>77</sup> In these cells, cGMP induces phosphorylation and degradation of I $\kappa$ B- $\alpha$ , and direct phosphorylation of I $\kappa$ B- $\alpha$  by PKG occurs in vitro.<sup>77</sup> NF- $\kappa$ B may also be activated by cGMP through direct PKG phosphorylation of p50 and p65 subunits.<sup>122</sup> In contrast, cytokine-induced NF- $\kappa$ B activation in human endothelial cells is inhibited by NO/cGMP and I $\kappa$ B- $\alpha$  is stabilized.<sup>123,124</sup> Natriuretic peptides and cGMP analogues also inhibit NF- $\kappa$ B activation during hepatic ischemia/reperfusion injury through stabilization of I $\kappa$ B; this correlates with cGMP induction of heat shock protein 70 and increased HSP70 association with I $\kappa$ B.<sup>125</sup>

**Calcineurin-Dependent Transcription Factors**

Calcineurin is a Ca<sup>2+</sup>-dependent phosphatase that dephosphorylates and thereby activates the transcription factors NF/AT, myocyte enhancer factor-2 (MEF-2), and Elk-1. In cardiomyocytes, PKG activation inhibits calcineurin-dependent NF/AT nuclear translocation, phenylephrine-in-

duced BNP promoter activity, and cell enlargement by interfering with Ca<sup>2+</sup> entry.<sup>30</sup> MEF-2-dependent transcription is also inhibited by cGMP/PKG.<sup>30</sup>

**Serum-Response Factor (SRF)**

Transcriptional activation of muscle-specific genes requires cooperation between SRF and other coactivators, such as myocardin or the myocardin-related protein MAL, and is regulated by RhoA-induced actin polymerization.<sup>126,127</sup> RhoA activation induces MAL translocation from the cytoplasm to the nucleus and stimulates SRF-dependent transcription.<sup>127</sup> Growth factor-induced transcriptional activation of immediate-early genes such as *c-fos* and *egr-1* also involves SRF, but on these promoters SRF cooperates with TCF; the SRF-TCF complex is regulated by MAP kinases, but is insensitive to RhoA signaling.<sup>92,128</sup>

NO/cGMP/PKG signaling can inhibit RhoA functions such as Ca<sup>2+</sup> sensitization of VSMC contractility and RhoA-dependent actin polymerization.<sup>19</sup> We showed that PKG inhibits SRF-dependent transcription by interfering with RhoA signaling in cardiomyocytes and VSMCs; PKG acts both upstream of RhoA, inhibiting serum- and G $\alpha_{13}$ -induced Rho activation, and downstream of RhoA, inhibiting steps distal to the Rho targets ROK, PKN, and PRK-2.<sup>5</sup> PKG also inhibits serum induction of vinculin mRNA, thus regulating an endogenous SRF target gene.<sup>5</sup>

During differentiation of SM cells, RhoA appears to play a dual role: (1) in undifferentiated cells, high RhoA activity delays differentiation by restricting SRF to the cytoplasm, whereas RhoA downregulation induces nuclear translocation of SRF and promotes myogenesis<sup>129</sup>; (2) in differentiated cells, SRF is associated with active, hyperacetylated chromatin, and RhoA activation by G protein-coupled receptors stimulates SRF-dependent transcription from many muscle-specific promoters.<sup>130</sup> Thus, PKG inhibition of RhoA in undifferentiated cells could provide a differentiation-promoting signal, while PKG inhibition of RhoA in differentiated cells may serve to restrict SM-specific promoter activity under conditions where RhoA is activated.

**Regulation of Gene Transcription Through cGMP Modulation of MAP Kinase Pathways**

Pathways that activate the MAP kinases Erk-1/2, p38, or JNK regulate gene expression through direct or indirect phosphorylation of multiple transcription factors, including TCF, CREB, ATF-2, and c-Jun (Figure 2).<sup>92</sup> Increased intracellular cGMP can lead to increased or decreased activity of all three MAP kinase pathways depending on cell type and growth conditions (online Table), and changes in MAP kinase activity may explain the effect of cGMP on many genes involved in cell proliferation, differentiation, or apoptosis. This has been documented in some cases, eg, the cGMP-mediated increase of metalloproteinase-13 and heme oxygenase-1 expression in endothelial cells is dependent on Erk-1/2 activation.<sup>131,132</sup>

In neonatal cardiomyocytes, ANF and cGMP analogues increase Erk-1/2 activity but have little effect on p38 MAP kinase or JNK activity.<sup>133</sup> In adult cardiomyocytes, NO increases both Erk-1/2 and p38 activity, but Erk-1/2 activa-

tion is cGMP-independent, whereas p38 activation is mediated by cGMP and PKG, with cAMP analogues having an opposing effect.<sup>134</sup>

In serum-starved aortic VSMCs, Erk-1/2 and JNK activities are stimulated by cGMP analogues in a PKG-dependent fashion.<sup>106</sup> However, platelet-derived growth factor-, epithelial growth factor-, and Ang II-stimulated Erk-1/2 activities in early passage VSMCs and in aortic strips are inhibited by NO, ANP/CNP, with several studies demonstrating that this effect is not due to cross-activation of PKA.<sup>46,47,135</sup> In fact, endothelium-derived NO appears to tonically inhibit the effect of endogenous Ang II on Erk-1/2 activity in the aorta.<sup>135</sup> Thus, cGMP-elevating agents may augment basal, but inhibit mitogen-stimulated Erk activity in primary VSMCs. Inhibition of mitogen-stimulated Erk activity has also been observed in mesangial cells and fibroblast-like cells.<sup>53,136,137</sup>

In endothelial cells, NO donors stimulate basal Erk-1/2 activity in a cGMP-dependent fashion; this effect is mimicked by cGMP analogues and in some cases by overexpression of constitutively active PKG.<sup>57,58,131,132</sup> Moreover, the mitogenic effect of VEGF on endothelial cells appears to be mediated by NO and cGMP, as VEGF-induced Erk-1/2 activation and proliferation is blocked by NOS and sGC inhibitors.<sup>57,58</sup>

In the classic MAP kinase pathway, growth factor receptors activate Ras, Raf kinases, MEK-1/2, and Erk-1/2 (Figure 2).<sup>92</sup> Although NO can directly activate Ras in vitro through S-nitrosylation of C<sup>118</sup>, the in vivo significance of this reaction remains to be confirmed.<sup>138</sup> In growth factor-stimulated VSMCs and BHK cells, NO/cGMP have no effect on Ras activity.<sup>46,53</sup> One group suggested that PKG can directly activate Raf-1 through phosphorylation of the kinase in a soluble Ras/Raf-1/PKG complex present in VEGF-stimulated human umbilical vein endothelial cells (HUVECs); however, specificity of the PKG antibody used was not demonstrated,<sup>57</sup> and HUVECs express very little or no detectable PKG by Western blotting,<sup>7</sup> making it doubtful that specific coimmunoprecipitation of PKG with Raf-1 and Ras would be detectable. Other investigators were unable to find Raf-1 activation by cGMP in cardiomyocytes where cGMP activated MEK-1/2 and Erk<sup>133</sup>; MEK-1/2 activation by cGMP/PKG has been confirmed in VSMCs.<sup>106</sup> PKG directly phosphorylates Ser<sup>43</sup> of Raf-1 in vitro and in vivo; this phosphorylation uncouples the Ras-Raf interaction and thereby prevents mitogen-induced Raf-1 activation.<sup>46,53</sup> A phosphorylation-deficient Raf-1(A<sup>43</sup>) mutant is insensitive to cGMP/PKG inhibition and protects cells from the inhibition of mitogen-induced Erk activity by cGMP.<sup>53</sup> Thus, Erk activation by cGMP in resting cells may or may not include activation of Ras and Raf kinases, but occurs at the level of MEK-1/2. On the other hand, Erk inhibition by cGMP/PKG in mitogen-stimulated cells may be explained by PKG phosphorylation of Raf-1 and by cGMP/PKG induction of the MAP kinase inhibitor MPK-1 (Figure 2 and Table 1).<sup>53</sup> In addition, cross-activation of PKA under conditions of high intracellular cGMP can lead to Erk pathway inhibition.<sup>4,54</sup> Cell type-specific activation of p38 or JNK by cGMP appears to involve activation of the

upstream kinases MEK-3 or MEKK-1 by PKG, respectively.<sup>71,139</sup>

### Posttranscriptional Regulation of Gene Expression by cGMP

Regulation of gene expression at the posttranscriptional level includes regulation of pre-mRNA splicing, mRNA stability, and translation. PKG phosphorylates splicing factor 1 (SF1) in vitro and in vivo, and thereby inhibits prespliceosome assembly; however, whether this phosphorylation results in alternative splice site selection or otherwise regulates RNA processing of specific genes is presently unknown.<sup>100</sup>

Elevation of intracellular cGMP can decrease mRNA levels of sGC- $\alpha_1$  and - $\beta_1$  subunits, iNOS, COX-2, TNF- $\alpha$ , and TGF- $\beta_3$  via message destabilization in VSMCs, mesangial cells, and cardiac fibroblasts.<sup>21,24,81,86,87,140</sup> Stability of sGC- $\alpha_1$  mRNA is regulated by the ubiquitous mRNA binding protein HuR, which binds to AU-rich elements in the 3'-untranslated region (UTR) and increases mRNA half-life; cGMP-elevating agents decrease expression and RNA binding of HuR, thereby destabilizing sGC- $\alpha_1$  mRNA.<sup>24</sup> The cGMP-induced mRNA destabilization requires transcription of an unknown factor(s)<sup>24,140</sup>; this could be an RNA-destabilizing protein or a factor involved in downregulation of HuR. HuR regulates mRNA stability of many different genes also regulated by cGMP, including iNOS, COX-2, TNF- $\alpha$ , VEGF, and cyclins A and D1.<sup>83,141</sup> Therefore, cGMP downregulation of HuR could potentially explain the cGMP downregulation of these mRNAs observed under some conditions. cGMP can also increase mRNA stability, as illustrated by the example of the potassium chloride cotransporter-3.<sup>142</sup>

Translational regulation by cGMP has been most thoroughly studied for the asialoglycoprotein receptor (ASGR), a hepatocellular surface lectin.<sup>143,144</sup> Increased intracellular cGMP shifts ASGR mRNA into a translationally active polysomal pool, whereas decreased intracellular cGMP causes a negative *trans*-acting factor (called COPI) to associate with the 5'-UTR of the ASGR mRNA, thereby preventing ribosomal scanning to the site of translational initiation.<sup>143,144</sup> cGMP induced phosphorylation of COPI in intact cells is prevented by PKG inhibition and correlates with increased ASGR synthesis; PKG-dependent phosphorylation of COPI may regulate association of the COPI/RNA complex.<sup>143</sup> Synthesis of insulin receptor subunits  $\alpha$  and  $\beta$  appears to be regulated by cGMP via a similar mechanism.<sup>143</sup>

### Conclusions

Although transcriptional regulation by cAMP has been intensely studied for decades, the regulation of gene expression by cGMP has only more recently been recognized. Many (patho)physiological processes in the cardiovascular system involve cGMP-induced changes in gene expression: (1) cGMP downregulates genes involved in the modulation of vascular tone, including sGC, PKG and rGC-A; (2) cGMP negatively regulates cardiac hypertrophy by inhibiting the expression of prohypertrophic genes such as muscle LIM protein; (3) cGMP/PKG modulate the VSMC phenotype and increase expression of differentiation-associated genes such

as SM-myosin heavy chain-2 and SM- $\alpha$ -actin; (4) cGMP regulation of cell proliferation correlates with the modulation of cell cycle-associated genes and growth factor synthesis; and (5) cGMP's effects on apoptosis appear to be mediated, at least in part, through regulation of Bcl-2-related genes. Gene expression profiling will likely contribute to the rapidly growing list of cGMP-regulated genes; however, the mechanisms of transcriptional and translational regulation by cGMP are only beginning to be understood. Some effects of cGMP on gene expression involve cross-talk with other signaling pathways, such as MAP kinase, calcineurin, and RhoA pathways; other effects of cGMP may be directly attributed to PKG phosphorylation of specific transcription factors, such as CREB and TFII-I.

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### References

- Beavo JA, Brunton LL. Cyclic nucleotide research: still expanding after half a century. *Nat Rev Mol Cell Biol*. 2002;3:710–718.
- Silberbach M, Roberts CT. Natriuretic peptide signaling: molecular and cellular pathways to growth regulation. *Cell Signal*. 2001;13:221–231.
- Lohmann SM, Vaandrager AB, Smolenski A, Walter U, DeJonge HR. Distinct and specific functions of cGMP-dependent protein kinases. *Trends Biochem Sci*. 1997;22:307–312.
- Cornwell TL, Arnold E, Boerth NJ, Lincoln TM. Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. *Am J Physiol*. 1994;267:C1405–C1413.
- Gudi T, Chen JC, Casteel DE, Seasholtz TM, Boss GR, Pilz RB. cGMP-dependent protein kinase inhibits serum response element-dependent transcription by inhibiting Rho activation and functions. *J Biol Chem*. 2002;277:37393–37382.
- Wollert KC, Fiedler B, Gambaryan S, Smolenski A, Heineke J, Butt E, Trautwein C, Lohmann SM, Drexler H. Gene transfer of cGMP-dependent protein kinase I enhances the antihypertrophic effects of nitric oxide in cardiomyocytes. *Hypertension*. 2002;39:87–92.
- Draijer R, Vaandrager AB, Nolte C, De Jonge HR, Walter U, Van Hinsbergh VWM. Expression of cGMP-dependent protein kinase I and phosphorylation of its substrate, vasodilator-stimulated phosphoprotein, in human endothelial cells of different origin. *Circ Res*. 1995;77:897–905.
- Esteve L, Lutz P, Thiriet N, Revel M, Aunis D, Zwiller J. cGMP-dependent protein kinase potentiates serotonin-induced Egr-1 binding activity in PC12 cells. *Cell Signal*. 2001;13:425–432.
- Andoh T, Chiueh CC, Chock B. cGMP-dependent protein kinase regulates the expression of thioredoxin and thioredoxin peroxidase-1 during hemoxyis in response to oxidative stress-induced apoptosis. *J Biol Chem*. 2003;278:885–890.
- Pfeifer A, Klatt P, Massberg S, Ny L, Sausbier M, Hirneib C, Wang G-X, Korth M, Aszodi A, Andersson K-E, Krombach F, Mayerhofer A, Ruth P, Fassler R, Hofmann F. Defective smooth muscle regulation in cGMP kinase I-deficient mice. *EMBO J*. 1998;17:3045–3051.
- Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JA, Berkowitz DE, Hare JM. NO regulates the heart by spatial confinement of NO synthase isoforms. *Nature*. 2002;416:337–339.
- Bogdan C. Nitric oxide and the regulation of gene expression. *Trends Cell Biol*. 2001;11:66–75.
- Pfeilschifter J, Eberhardt W, Beck KF. Regulation of gene expression by nitric oxide. *Pflügers Arch*. 2001;442:479–486.
- Burkhardt M, Glazova M, Gambaryan S, Vollkommer T, Butt E, Bader B, Heermeier K, Lincoln TM, Walter U, Palmethofer A. KT5823 inhibits cGMP-dependent protein kinase activity in vitro but not in intact human platelets and rat mesangial cells. *J Biol Chem*. 2000;275:33536–33541.
- Wang EQ, Lee WI, Brazeau D, Fung HL. cDNA microarray analysis of vascular gene expression after nitric oxide donor infusions in rats: implications for nitrate tolerance mechanisms. *AAPS Pharm Sci*. 2002;4:E10.
- Heineke J, Kempf T, Kraft T, Hilfiker A, Morawietz H, Scheubel RJ, Caroni P, Lohmann SM, Drexler H, Wollert KC. Downregulation of cytoskeletal muscle LIM protein by nitric oxide: impact on cardiac myocyte hypertrophy. *Circulation*. 2003;107:1424–1432.
- Cappola TP, Cope L, Cernetic A, Barouch LA, Minhas K, Irizarry RA, Parmigiani G, Durrani S, Lavoie T, Hoffman EP, Ye SQ, Garcia G, Hare JM. Deficiency of different NO synthase isoforms activates divergent transcriptional programs in cardiac hypertrophy. *Physiol Genomics*. 2003;14:25–34.
- Wang D, Oparil S, Feng JA, Li P, Perry G, Chen LB, Dai M, John SW, Chen Y-F. Effect of pressure overload on extracellular matrix expression in the heart of the atrial natriuretic peptide-null mouse. *Hypertension*. 2003;42:88–95.
- Sauzeau V, Jeune HL, Cario-Toumaniantz C, Smolenski A, Lohmann SM, Bertoglio J, Chardin P, Pacaud P, Loirand G. Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced  $\text{Ca}^{2+}$  sensitization of contraction in vascular smooth muscle. *J Biol Chem*. 2000;275:21722–21729.
- Sauzeau V, Rolli-Derkinderen M, Marionneau C, Loirand G, Pacaud P. RhoA expression is controlled by NO through cGMP-dependent protein kinase activation. *J Biol Chem*. 2003;278:9472–9480.
- Filipov G, Bloch DB, Bloch KD. Nitric oxide decreases stability of mRNAs encoding soluble guanylate cyclase subunits in rat pulmonary artery smooth muscle cells. *J Clin Invest*. 1997;100:942–948.
- Soff GA, Cornwell TL, Cundiff DL, Gately S, Lincoln TM. Smooth muscle cell expression of type I cyclic GMP-dependent protein kinase is suppressed by continuous exposure to nitrovasodilators, theophylline, cyclic GMP, and cyclic AMP. *J Clin Invest*. 1997;100:2580–2587.
- Cao L, Wu J, Gardner DG. Atrial natriuretic peptide suppresses the transcription of its guanylyl cyclase-linked receptor. *J Biol Chem*. 1995;270:24891–24897.
- Klöß S, Furneaux H, Mülsch A. Post-transcriptional regulation of soluble guanylyl cyclase expression in rat aorta. *J Biol Chem*. 2003;278:2377–2383.
- Yamashita T, Kawashima S, Ohashi Y, Ozaki M, Rikitake Y, Inoue N, Hirata K, Akita H, Yokoyama M. Mechanisms of reduced nitric oxide/cGMP-mediated vasorelaxation in transgenic mice overexpressing endothelial nitric oxide synthase. *Hypertension*. 2000;36:97–102.
- Sellak H, Yang X, Cao X, Cornwell T, Soff GA, Lincoln T. Sp1 transcription factor as a molecular target for NO and cyclic nucleotide-mediated suppression of cGMP-dependent protein kinase-I $\alpha$  expression in vascular smooth muscle cells. *Circ Res*. 2002;90:405–412.
- Vera N, Tse MY, Watson JD, Sarda S, Steinhilber ME, John SW, Flynn TG, Pang SC. Altered expression of natriuretic peptide receptors in proANP gene disrupted mice. *Cardiovasc Res*. 2000;46:595–603.
- Sugden PH, Clerk A. Cellular mechanisms of cardiac hypertrophy. *J Mol Med*. 1998;76:725–746.
- Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Inhibitory regulation of hypertrophy by endogenous atrial natriuretic peptide in cultured cardiac myocytes. *Hypertension*. 2000;35:19–24.
- Fiedler B, Lohmann SM, Smolenski A, Linnemuller S, Pieske B, Schroder F, Molkentin JD, Drexler H, Wollert KC. Inhibition of calcineurin-NFAT hypertrophy signaling by cGMP-dependent protein kinase type I in cardiac myocytes. *Proc Natl Acad Sci U S A*. 2002;99:11363–11368.
- Lincoln TM, Dey N, Sellak H. cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. *J Appl Physiol*. 2001;91:1421–1430.
- Boerth NJ, Dey NB, Cornwell TL, Lincoln TM. Cyclic GMP-dependent protein kinase regulates vascular smooth muscle cell phenotype. *J Vasc Res*. 1997;34:245–259.
- Dey NB, Boerth NJ, Murphy-Ullrich JE, Chang P-L, Prince CW, Lincoln TM. Cyclic GMP-dependent protein kinase inhibits osteopontin and thrombospondin production in rat aortic smooth muscle cells. *Circ Res*. 1998;82:139–146.
- Lincoln TM, Dey NB, Boerth NJ, Cornwell TL, Soff GA. Nitric oxide-cyclic GMP pathway regulates vascular smooth muscle cell phenotypic modulation: implications in vascular diseases. *Acta Physiol Scand*. 1998;164:507–513.
- Chen Q, Chen YE, Tamura K, Pratt R. Identification of a cGMP responsive element in smooth muscle myosin promoter. *FASEB J*. 2000;14:A406. Abstract.

36. Doi K, Ikeda T, Itoh H, Ueyama K, Hosoda K, Ogawa Y, Yamashita J, Chun TH, Inoue M, Matsutsugu K, Sawada N, Fukunaga Y, Saito T, Sone M, Yamahara K, Kook H, Komeda M, Ueda M, Nakao K. C-type natriuretic peptide induces redifferentiation of vascular smooth muscle cells with accelerated reendothelialization. *Arterioscler Thromb Vasc Biol*. 2001;21:930–936.
37. Itoh S, Katoh Y, Konishi H, Takaya N, Kimura T, Periasamy M, Yamaguchi H. Nitric oxide regulates smooth-muscle-specific myosin heavy chain gene expression at the transcriptional level-possible role of SRF and YY1 through CArG element. *J Mol Cell Cardiol*. 2001;33:95–107.
38. Chen Q, Tang HC, Dzau VJ, Pratt RE. Genomic analysis of human smooth muscle cell differentiation using high density microarrays. *Circulation*. 2000;102:11–226. Abstract.
39. Sinnaeve P, Chiche JD, Gillijns H, Van Pelt N, Wirthlin D, Van De Werf F, Collen D, Bloch KD, Janssens S. Overexpression of a constitutively active protein kinase G mutant reduces neointima formation and in-stent restenosis. *Circulation*. 2002;105:2911–2916.
40. Anderson PG, Boerth NJ, Liu M, McNamara DB, Cornwell TL, Lincoln TM. Cyclic GMP-dependent protein kinase expression in coronary arterial smooth muscle in response to balloon catheter injury. *Arterioscler Thromb Vasc Biol*. 2000;20:2192–2197.
41. Monks D, Lange V, Silber RE, Markert T, Walter U, Nehls V. Expression of cGMP-dependent protein kinase I and its substrate VASP in neointimal cells of the injured rat carotid artery. *Eur J Clin Invest*. 1998;28:416–423.
42. Regan CP, Adam PJ, Madsen CS, Owens GK. Molecular mechanisms of decreased smooth muscle differentiation marker expression after vascular injury. *J Clin Invest*. 2000;106:1139–1147.
43. Sinnaeve P, Chiche JD, Nong Z, Varenne O, Van Pelt N, Gillijns H, Collen D, Bloch KD, Janssens S. Soluble guanylate cyclase  $\alpha_1$  and  $\beta_1$  gene transfer increases NO responsiveness and reduces neointima formation after balloon injury in rats via antiproliferative and antimigratory effects. *Circ Res*. 2001;88:103–109.
44. Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, Sessa WC. Direct evidence for the importance of endothelium-derived NO in vascular remodeling. *J Clin Invest*. 1998;101:731–736.
45. Chiche J-D, Schlusmeyer SM, Bloch DB, de la Monte SM, Roberts JD, Filippov G, Janssens SP, Rosenzweig A, Bloch KD. Adenovirus-mediated gene transfer of cGMP-dependent protein kinase increases the sensitivity of cultured vascular smooth muscle cells to the antiproliferative and pro-apoptotic effects of nitric oxide/cGMP. *J Biol Chem*. 1998;273:34263–34271.
46. Yu S-M, Hung L-M, Lin C-C. cGMP-elevating agents suppress proliferation of vascular smooth muscle cells by inhibiting the activation of epidermal growth factor signaling pathway. *Circulation*. 1997;5:1269–1277.
47. Hutchinson G, Trindade PT, Cunan DB, Wu WCF, Pratt RE. Mechanisms of natriuretic-peptide-induced growth inhibition of vascular smooth muscle cells. *Cardiovasc Res*. 1997;35:158–167.
48. Fukumoto S, Koyama H, Hosoi M, Yamakawa K, Tanaka S, Morii H, Nishizawa Y. Distinct role of cAMP and cGMP in the cell cycle control of vascular smooth muscle cells: cGMP delays cell cycle transition through suppression of cyclin D1 and cyclin-dependent kinase 4 activation. *Circ Res*. 1999;85:985–991.
49. Gu M, Lynch J, Brecher P. Nitric oxide increases p21<sup>Waf1/Cip1</sup> expression by a cGMP-dependent pathway that includes activation of extracellular signal-regulated kinase and p70<sup>S6k</sup>. *J Biol Chem*. 2000;275:11389–11396.
50. Hanada S, Terada Y, Inoshita S, Sasaki S, Lohmann SM, Smolenski A, Marumo F. Overexpression of protein kinase G using adenovirus inhibits cyclin E transcription and mesangial cell cycle. *Am J Physiol Renal Physiol*. 2001;280:F851–F859.
51. Takizawa T, Gu M, Chobanian AV, Brecher P. Effect of nitric oxide on DNA replication induced by angiotensin II in rat cardiac fibroblasts. *Hypertension*. 1997;30:1035–1040.
52. Fujisaki H, Ito H, Hirata Y, Tanaka M, Hata M, Lin M, Adachi S, Akimoto H, Marumo F, Hiroe M. Natriuretic peptides inhibit angiotensin II-induced proliferation of rat cardiac fibroblasts by blocking endothelin-1 gene expression. *J Clin Invest*. 1995;96:1059–1065.
53. Suhasini M, Li H, Lohmann SM, Boss GR, Pilz RB. Cyclic-GMP-dependent protein kinase inhibits the Ras/mitogen-activated protein kinase pathway. *Mol Cell Biol*. 1998;18:6983–6994.
54. Osinski MT, Rauch BH, Schror K. Antimitogenic actions of organic nitrates are potentiated by sildenafil and mediated via activation of protein kinase A. *Mol Pharmacol*. 2001;59:1044–1050.
55. Mitsutomi N, Akashi C, Odagiri J, Matsumura Y. Effects of endogenous and exogenous nitric oxide on endothelin-1 production in cultured vascular endothelial cells. *Eur J Pharmacol*. 1999;364:65–73.
56. Zhang R, Wang L, Zhang L, Chen J, Zhu Z, Zhan Z. Nitric oxide enhances angiogenesis via the synthesis of vascular endothelial growth factor and cGMP after stroke in the rat. *Circ Res*. 2003;92:308–313.
57. Hood J, Granger HJ. Protein kinase G mediates vascular endothelial growth factor-induced Raf-1 activation and proliferation in human endothelial cells. *J Biol Chem*. 1998;273:23504–23508.
58. Parenti A, Morbidelli L, Cui X-L, Douglas JG, Hood JD, Granger HJ, Ledda F, Ziche M. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase 1/2 activation in postcapillary endothelium. *J Biol Chem*. 1998;273:4220–4226.
59. Begum N, Ragolia L, Rienzie J, McCarthy M, Duddy N. Regulation of mitogen-activated protein kinase phosphatase-1 induction by insulin in vascular smooth muscle cells. *J Biol Chem*. 1998;273:25164–25170.
60. Sugimoto T, Haneda M, Togawa M, Isono M, Shikano T, Araki S, Nakagawa T, Kashiwagi A, Guan K-L, Kikkawa R. Atrial natriuretic peptide induces the expression of MKP-1, a mitogen-activated protein kinase phosphatase, in glomerular mesangial cells. *J Biol Chem*. 1996;271:544–547.
61. Kierner AK, Weber NC, Furst R, Bildner N, Kulhanek-Heinze S, Vollmar AM. Inhibition of p38 MAPK activation via induction of MKP-1: atrial natriuretic peptide reduces TNF- $\alpha$ -induced actin polymerization and endothelial permeability. *Circ Res*. 2002;90:874–881.
62. Kronemann N, Nockher WA, Busse R, Schini-Kerth VB. Growth-inhibitory effect of cyclic GMP- and cyclic AMP-dependent vasodilators on rat vascular smooth muscle cells: effect on cell cycle and cyclin expression. *Br J Pharmacol*. 1999;126:349–357.
63. Dulak J, Jozkowicz A, Dembinska-Kiec A, Guevara I, Zdzienicka A, Zmudzinska-Grochot D, Florek I, Wojtowicz A, Szuba A, Cooke JP. NO induces the synthesis of vascular endothelial growth factor by rat vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2000;20:659–666.
64. Chin K, Kurashima Y, Ogura T, Tajiri H, Yoshida S, Esumi H. Induction of vascular endothelial growth factor by nitric oxide in human glioblastoma and hepatocellular carcinoma cells. *Oncogene*. 1997;15:437–442.
65. Liu Y, Christou H, Morita T, Laughner E, Semenza GL, Kourembanas S. CO and NO suppress the hypoxic induction of VEGF via the 5' enhancer. *J Biol Chem*. 1998;273:15257–15262.
66. Tsurumi Y, Murohara T, Krasinski K, Chen D, Witzenbichler B, Kearney M, Couffinhal T, Isner JM. Reciprocal relation between VEGF and NO in the regulation of endothelial integrity. *Nat Med*. 1997;3:879–886.
67. Wu CF, Bishopric NH, Pratt RE. Atrial natriuretic peptide induces apoptosis in neonatal rat cardiac myocytes. *J Biol Chem*. 1997;272:14860–14866.
68. Pollman MJ, Yamada T, Horiuchi M, Gibbons GH. Vasoactive substances regulate vascular smooth muscle cell apoptosis. *Circ Res*. 1996;79:748–756.
69. Suenobu N, Shichiri M, Iwashina M, Marumo F, Hirata Y. Natriuretic peptides and nitric oxide induce endothelial apoptosis via a cGMP-dependent mechanism. *Arterioscler Thromb Vasc Biol*. 1999;19:140–146.
70. Thompson WJ, Piazza GA, Li H, Liu L, Fetter J, Zhu B, Sperl G, Ahnen D, Pamukcu R. Exisulind induction of apoptosis involves guanosine 3',5'-cyclic monophosphate phosphodiesterase inhibition, protein kinase G activation, and attenuated  $\beta$ -catenin. *Cancer Res*. 2000;60:3338–3342.
71. Soh JW, Mao Y, Liu L, Thompson WJ, Pamukcu R, Weinstein IB. Protein kinase G activates the JNK1 pathway via phosphorylation of MEKK1. *J Biol Chem*. 2001;276:16406–16410.
72. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. *Nat Cell Biol*. 2002;4:E131–E136.
73. Ciani E, Guidi S, Bartsaghi R, Contestabile A. Nitric oxide regulates cGMP-dependent cAMP-responsive element binding protein phosphorylation and Bcl-2 expression in cerebellar neurons. *J Neurochem*. 2002;82:1282–1289.
74. Zamora R, Alarcon L, Vodovotz Y, Betten B, Kim PK, Gibson KF, Billiar TR. Nitric oxide suppresses the expression of Bcl-2 binding protein BNIP3 in hepatocytes. *J Biol Chem*. 2001;276:46887–46895.
75. Genaro AM, Hortelano S, Alvarez A, Martinez C, Bosca L. Splenic B lymphocyte programmed cell death is prevented by nitric oxide release

- through mechanisms involving sustained Bcl-2 levels. *J Clin Invest.* 1995;95:1884–1890.
76. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, Carruba MO. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science.* 2003;299:896–899.
  77. Kalra D, Baumgarten G, Dibbs Z, Seta Y, Sivasubramanian N, Mann DL. Nitric oxide provokes tumor necrosis factor- $\alpha$  expression in adult feline myocardium through a cGMP-dependent pathway. *Circulation.* 2000;102:1302–1307.
  78. Muhl H, Pfeilschifter J. Amplification of nitric oxide synthase expression by nitric oxide in interleukin 1 $\beta$ -stimulated rat mesangial cells. *J Clin Invest.* 1995;95:1941–1946.
  79. Yamamoto K, Ikeda U, Shimada K. Natriuretic peptides modulate nitric oxide synthesis in cytokine-stimulated cardiac myocytes. *J Mol Cell Cardiol.* 1997;29:2375–2382.
  80. Perez-Sala D, Lamas S. Regulation of cyclooxygenase-2 expression by nitric oxide in cells. *Antioxid Redox Signal.* 2001;3:231–248.
  81. Perez-Sala D, Cernuda-Morollon E, Diaz-Cazorla M, Rodriguez-Pascual F, Lamas S. Posttranscriptional regulation of human iNOS by the NO/cGMP pathway. *Am J Physiol Renal Physiol.* 2001;280:F466–F473.
  82. Diaz-Cazorla M, Perez-Sala D, Lamas S. Dual effect of nitric oxide donors on cyclooxygenase-2 expression in human mesangial cells. *J Am Soc Nephrol.* 1999;10:943–952.
  83. Brennan CM, Steitz JA. HuR and mRNA stability. *Cell Mol Life Sci.* 2001;58:266–277.
  84. Inoue T, Fukuo K, Nakahashi T, Hata S, Morimoto S, Ogihara T. cGMP upregulates nitric oxide synthase expression in vascular smooth muscle cells. *Hypertension.* 1995;25:711–714.
  85. Gong J-H, Renz H, Sprenger H, Nain M, Gerns D. Enhancement of tumor necrosis factor- $\alpha$  gene expression by low doses of prostaglandin E<sub>2</sub> and cyclic GMP. *Immunobiology.* 1990;182:44–55.
  86. Sinha B, Eigler A, Baumann KH, Greten TF, Moeller J, Endres S. Nitric oxide downregulates tumour necrosis factor in mRNA in RAW 264.7 cells. *Res Immunol.* 1998;149:139–150.
  87. Kierner AK, Vollmar AM. Autocrine regulation of inducible nitric-oxide synthase in macrophages by atrial natriuretic peptide. *J Biol Chem.* 1998;273:13444–13451.
  88. Tetsuka T, Daphna-Iken D, Miller BW, Guan Z, Baier LD, Morrison AR. Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. *J Clin Invest.* 1996;97:2051–2056.
  89. Cheng H-F, Wang J-L, Zhang M-Z, McKanna JA, Harris RC. Nitric oxide regulates renal cortical cyclooxygenase-2 expression. *Am J Physiol Renal Physiol.* 2000;278:F122–F129.
  90. Bouchie JL, Hansen H, Feener EP. Natriuretic factors and nitric oxide suppress plasminogen activator inhibitor-1 expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 1998;18:1771–1779.
  91. Yoshizumi M, Tsuji H, Nishimura H, Masuda H, Kunieda Y, Kawano H, Kimura S, Sugano T, Kitamura H, Nakagawa K, Nakagawa M. Natriuretic peptides regulate the expression of tissue factor and PAI-1 in endothelial cells. *Thromb Haemost.* 1999;82:1497–1503.
  92. Hazzalin CA, Mahadevan LC. MAPK-regulated transcription: a continuously variable gene switch? *Nat Rev Mol Cell Biol.* 2002;3:30–40.
  93. Shaywitz AJ, Greenberg ME. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem.* 1999;68:821–861.
  94. Lu F, Kandel ER, Hawkins RD. Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. *J Neurosci.* 1999;19:10250–10261.
  95. Gudi T, Casteel DE, Vinson C, Boss GR, Pilz RB. NO activation of *fos* promoter elements requires nuclear translocation of G-kinase I and CREB phosphorylation but is independent of MAP kinase activation. *Oncogene.* 2000;19:6324–6333.
  96. Gudi T, Huvar I, Meinecke M, Lohmann SM, Boss GR, Pilz RB. Regulation of gene expression by cGMP-dependent protein kinase. *J Biol Chem.* 1996;271:4597–4600.
  97. Collins SP, Uhler MD. Cyclic AMP- and cyclic GMP-dependent protein kinases differ in their regulation of cyclic AMP response element-dependent gene transcription. *J Biol Chem.* 1999;274:8391–8404.
  98. Matthews RP, Guthrie CR, Wailes LM, Zhao X, Means AR, McKnight GS. Calcium/calmodulin-dependent protein kinase types II and IV differentially regulate CREB-dependent gene expression. *Mol Cell Biol.* 1994;14:6107–6116.
  99. Gudi T, Lohmann SM, Pilz RB. Regulation of gene expression by cGMP-dependent protein kinase requires nuclear translocation of the kinase: identification of a nuclear localization signal. *Mol Cell Biol.* 1997;17:5244–5254.
  100. Wang X, Bruderer S, Rafi Z, Xue J, Milburn PJ, Krämer A, Robinson PJ. Phosphorylation of splicing factor SF1 on Ser20 by cGMP-dependent protein kinase regulates spliceosome assembly. *EMBO J.* 1999;18:4549–4559.
  101. Wyatt TA, Lincoln TM, Pryzwansky KB. Vimentin is transiently co-localized with and phosphorylated by cyclic GMP-dependent protein kinase in formyl-peptide-stimulated neutrophils. *J Biol Chem.* 1991;266:21274–21280.
  102. Pryzwansky KB, Kidao S, Wyatt TA, Reed W, Lincoln TM. Localization of cyclic GMP-dependent protein kinase in human mononuclear phagocytes. *J Leukoc Biol.* 1995;57:670–678.
  103. Browning DD, McShane M, Marty C, Ye RD. Functional analysis of type 1- $\alpha$  cGMP-dependent protein kinase using green fluorescent fusion proteins. *J Biol Chem.* 2001;276:13039–13048.
  104. Feil R, Gappa N, Rutz M, Schlossmann J, Rose CR, Konnerth A, Brummer S, Kuhbandner S, Hofmann F. Functional reconstitution of vascular smooth muscle cells with cGMP-dependent protein kinase I isoforms. *Circ Res.* 2002;90:1080–1086.
  105. Casteel DE, Zhuang S, Gudi T, Tang J, Vuica M, Desiderio S, Pilz RB. cGMP-dependent protein kinase I $\beta$  physically and functionally interacts with the transcriptional regulator TFII-I. *J Biol Chem.* 2002;277:32003–32014.
  106. Komalavilas P, Shah PK, Jo H, Lincoln TM. Activation of mitogen-activated protein kinase pathways by cyclic GMP and cyclic GMP-dependent protein kinase in contractile vascular smooth muscle cells. *J Biol Chem.* 1999;274:34301–34309.
  107. Roy AL. Biochemistry and biology of the inducible multifunctional transcription factor TFII-I. *Gene.* 2001;274:1–13.
  108. Parker R, Phan T, Baumeister P, Roy B, Cheriya V, Roy AL, Lee AS. Identification of TFII-I as the endoplasmic reticulum stress response element binding factor ERSF: its autoregulation by stress and interaction with ATF6. *Mol Cell Biol.* 2001;21:3220–3233.
  109. Wen Y-D, Cress WD, Roy AL, Seto E. Histone deacetylase 3 binds to and regulates the multifunctional transcription factor TFII-I. *J Biol Chem.* 2003;278:1841–1847.
  110. Thiriet N, Esteve L, Aunis D, Zwiler J. Immediate early gene induction by natriuretic peptides in PC12 pheochromocytoma and C6 glioma cells. *Mol Neurosci.* 1997;8:399–402.
  111. Pilz RB, Suhasini M, Idriss S, Meinke JL, Boss GR. Nitric oxide and cGMP analogs activate transcription from AP-1-responsive promoters in mammalian cells. *FASEB J.* 1995;9:552–558.
  112. Wu J, Fang L, Lin Q, Willis WD. Fos expression is induced by increased nitric oxide release in rat spinal cord dorsal horn. *Neuroscience.* 2000;96:351–357.
  113. Pawliczak R, Cowan MJ, Huang X, Nanavaty UB, Alsaaty S, Logan C, Shelhamer JH. p11 expression in human bronchial epithelial cells is increased by nitric oxide in a cGMP-dependent pathway involving protein kinase G activation. *J Biol Chem.* 2001;276:44613–44621.
  114. Gertzberg N, Clements R, Jaspers I, Ferro TJ, Neumann P, Flescher E, Johnson A. TNF- $\alpha$ -induced activating protein-1 activity is modulated by nitric oxide-mediated protein kinase G activation. *Am J Respir Cell Mol Biol.* 2000;22:105–115.
  115. Klatt P, Molina EP, Lamas S. Nitric oxide inhibits c-Jun DNA binding by specifically targeted S-glutathionylation. *J Biol Chem.* 1999;274:15857–15864.
  116. Nikitovic D, Holmgren A, Spyrou G. Inhibition of AP-1 DNA binding by nitric oxide involving conserved cysteine residues in Jun and Fos. *Biochem Biophys Res Commun.* 1998;242:109–112.
  117. Idriss SD, Gudi T, Casteel DE, Kharitonov VG, Pilz RB, Boss GR. Nitric oxide regulation of gene transcription via soluble guanylate cyclase and type I cGMP-dependent protein kinase. *J Biol Chem.* 1999;274:9489–9493.
  118. Walsh K, Takahashi A. Transcriptional regulation of vascular smooth muscle cell phenotype. *Z Kardiol.* 2001;90(suppl 3):12–16.
  119. Cibelli G, Policastro V, Rossler OG, Thiel G. Nitric oxide-induced programmed cell death in human neuroblastoma cells is accompanied by the synthesis of Egr-1, a zinc finger transcription factor. *J Neurosci Res.* 2002;67:450–460.
  120. Yamashita J, Itoh H, Ogawa Y, Tamura N, Takaya K, Igaki T, Doi K, Chun TH, Inoue M, Masatsugu K, Nakao K. Opposite regulation of Gax

- homeobox expression by angiotensin II and C-type natriuretic peptide. *Hypertension*. 1997;29:381–387.
121. Tak PP, Firestein GS. NF- $\kappa$ B: a key role in inflammatory diseases. *J Clin Invest*. 2001;107:7–11.
  122. He B, Weber GF. Phosphorylation of NF- $\kappa$ B proteins by cGMP-dependent kinase. *Eur J Biochem*. 2003;270:2174–2185.
  123. Kiemer AK, Weber NC, Vollmar AM. Induction of I $\kappa$ B atrial natriuretic peptide as a regulator of the NF- $\kappa$ B pathway. *Biochem Biophys Res Commun*. 2002;295:1068–1076.
  124. Tsao PS, Buitrago R, Chan JR, Cooke JP. Fluid flow inhibits endothelial adhesiveness: nitric oxide and transcriptional regulation of VCAM-1. *Circulation*. 1996;94:1682–1689.
  125. Kiemer AK, Gerbes AL, Bilzer M, Vollmar AM. The atrial natriuretic peptide and cGMP: novel activators of the heat shock response in rat livers. *Hepatology*. 2002;35:88–94.
  126. Yoshida T, Sinha S, Dandre F, Wamhoff BR, Hoofnagle MH, Kremer BE, Wang DZ, Olson EN, Owens GK. Myocardin is a key regulator of CArG-dependent transcription of multiple smooth muscle marker genes. *Circ Res*. 2003;92:856–864.
  127. Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell*. 2003;113:329–342.
  128. Murai K, Treisman R. Interaction of serum response factor (SRF) with the Elk-1 B box inhibits RhoA-Actin signaling to SRF and potentiates transcriptional activation by Elk-1. *Mol Cell Biol*. 2002;22:7083–7092.
  129. Beqaj S, Jakkaraju S, Mattingly RR, Pan D, Schuger L. High RhoA activity maintains the undifferentiated mesenchymal cell phenotype, whereas RhoA down-regulation by laminin-2 induces smooth muscle myogenesis. *J Cell Biol*. 2002;156:893–903.
  130. Manabe I, Owens GK. Recruitment of serum response factor and hyperacetylation of histones at smooth muscle-specific regulatory regions during differentiation of a novel P19-derived in vitro smooth muscle differentiation system. *Circ Res*. 2001;88:1127–1134.
  131. Zaragoza C, Soria E, Lopez E, Browning D, Balbin M, Lopez-Otin C, Lamas S. Activation of the MAP-kinase extracellular signal-regulated kinase-1 and -2 by the nitric oxide-cGMP-cGMP-dependent protein kinase axis regulates the expression of matrix metalloproteinase 13 in vascular endothelial cells. *Mol Pharmacol*. 2002;62:927–935.
  132. Kiemer AK, Bildner N, Weber NC, Vollmar AM. Characterization of heme oxygenase-1 (heat shock protein 32) induction by atrial natriuretic peptide in human endothelial cells. *Endocrinology*. 2003;144:802–812.
  133. Silberbach M, Gorenc T, Hershberger RE, Stork PJ, Steyger PS, Roberts CT Jr. Extracellular signal-regulated protein kinase activation is required for the anti-hypertrophic effect of atrial natriuretic factor in neonatal rat ventricular myocytes. *J Biol Chem*. 1999;274:24858–24864.
  134. Kim SO, Xu Y, Katz S, Pelech S. Cyclic GMP-dependent and -independent regulation of MAP kinases by sodium nitroprusside in isolated cardiomyocytes. *Biochim Biophys Acta*. 2000;1496:277–284.
  135. Kubo T, Saito E, Hanada M, Kambe T, Hagiwara Y. Evidence that angiotensin II, endothelins and nitric oxide regulate mitogen-activated protein kinase activity in rat aorta. *Eur J Pharmacol*. 1998;347:337–346.
  136. Pandey KN, Nguyen HT, Li M, Boyle JW. Natriuretic peptide receptor-A negatively regulates mitogen-activated protein kinase and proliferation of mesangial cells: role of cGMP-dependent protein kinase. *Biochem Biophys Res Commun*. 2000;271:374–379.
  137. Chrisman TD, Garbers DL. Reciprocal antagonism coordinates C-type natriuretic peptide and mitogen-signaling pathways in fibroblasts. *J Biol Chem*. 1999;274:4293–4299.
  138. Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, Quilliam LA. A molecular redox switch on p21<sup>ras</sup>: structural basis for the nitric oxide-p21<sup>ras</sup> interaction. *J Biol Chem*. 1997;272:4323–4326.
  139. Browning DD, McShane MP, Marty C, Ye RD. Nitric oxide activation of p38 mitogen-activated protein kinase in 293T fibroblasts requires cGMP-dependent protein kinase. *J Biol Chem*. 2000;275:2811–2816.
  140. Abdelaziz N, Colombo F, Mercier I, Calderone A. Nitric oxide attenuates the expression of transforming growth factor- $\beta_3$  mRNA in rat cardiac fibroblasts via destabilization. *Hypertension*. 2001;38:261–266.
  141. Rodriguez-Pascual F, Hausding M, Ihrig-Bieder I, Furneaux H, Levy AP, Forstermann U, Kleinert H. Complex contribution of the 3'-untranslated region to the expressional regulation of the human inducible nitric-oxide synthase gene: involvement of the RNA-binding protein HuR. *J Biol Chem*. 2000;275:26040–26049.
  142. Di Fulvio M, Lincoln TM, Lauf PK, Adragna NC. Protein kinase G regulates potassium chloride cotransporter-3 expression in primary cultures of rat vascular smooth muscle cells. *J Biol Chem*. 2001;276:21046–21052.
  143. De La Vega LA, Stockert RJ. Regulation of the insulin and asialoglycoprotein receptors via cGMP-dependent protein kinase. *Am J Physiol Cell Physiol*. 2000;279:C2037–C2042.
  144. De La Vega LA, Stockert RJ. The cytoplasmic coatmer protein COPI: a potential translational regulator. *J Biol Chem*. 1999;274:31135–31138.